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6-HYDROXYCYANIDIN 3-MALONYLGLUCOSIDE FROM THE FLOWERS OF *ALSTROEMERIA* 'TIARA'

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Abstract----- A novel malonylanthocyanin was isolated from the flowers of *Alstroemeria* 'Tiara' and determined to be 6-hydroxycyanidin 3-*O*-[6-*O*-malonyl-β-D-glucopyranoside].

From the flowers of *Alstroemeria* cultivars, at least, seven anthocyanins have been isolated and determined to be 3-glucoside of 6-hydroxycyanidin, [1] 3-rutinosides of delphinidin, cyanidin, 6-hydroxydelphinidin and 6-hydroxycyanidin, [1-4] and 3-malonylglucosides of delphinidin and cyanidin[3,4] by Nϕrbaek and by us, independently. During the course of our further studies directed forward the structure determination of flower colors of *Alstroemeria* species, we could isolate a novel anthocyanin,

and here we wish to report the structure elucidation of a novel malonylated anthocyanin along with three known anthocyanins in the flowers of *Alstroemeria* 'Tiara'.

The red cultivars of *Alstroemeria* 'Tiara' were obtained at the flower market in Sapporo. The flowers exhibited red colors (Red 43A by R.H.S. color chart, chromaticity value (b/a=0.65). The fresh perianthes of these plants were dried overnighit at 37^{o}C and kept in the refrigerator at 10 ^{o}C. Dried perianthes (*ca*. 50 g) of *Alstroemeria* 'Tiara' were immersed in 10% HOAc-MeOH (1 L, HOAc-MeOH, 1:9) at room temperature overnight. The pigments were then extracted with MAW (MeOH/HOAc/H_2O, 4:1:5), and the MAW extract was concentrated to *ca.* 10 mL, and purified by preparative HPLC. Preparative HPLC was run on a Waters C18 (19ϕ x 150 mm) column at 40 ^{o}C with a flow rate of 4 mL/min and monitored at 530 nm for anthocyanins. A solvent system used was linear gradient elution for 15 min from 40 to 60% solvent B (1.5% H_3PO_4, 20% HOAc, 25% MeCN in H_2O) in solvent A (1.5% H_3PO_4 in H_2O). The pigment fractions were evaporated in vacuo to dryness. The residues were dissolved in a small amount of 10% HOAc-MeOH followed by addition of an excess Et_2O, and then dried to give four pigment powders. Finally, pigment (**1**)[5] (*ca*. 2 mg), pigment (**2**)[6] (*ca.* 5 mg), pigment (**3**)[7] (*ca.* 1 mg) and pigment (**4**)[8] (*ca.* 3 mg) were obtained, respectively.

By acid hydrolysis, pigment (**1**) gave 6-hydroxycyanidin as the anthocyanidin, and glucose as the sugar residue. Similar treatment of pigment (**2**) afforded the same anthocyanidin, 6-hydroxycyanidin together with glucose and rhamnose as the sugar residues. On the other hand, cyanidin was isolated as the anthocyanidin by acid hydrolysis of pigments (**3**) and (**4**), where glucose was obtained from the former pigment (**3**), and glucose and rhamnose were found in the later pigment (**4**). Among these four pigments, three (**2**, **3** and **4**) of them were already known, and identified based on their spectroscopic data and also by direct comparison with the authentic samples, as 6-hydroxycyanidin 3-rutinoside, cyanidin 3-malonylglucoside, and cyanidin 3-rutinoside, respectively. Pigment (**1**), however, seemed

to be a new anthocyanin, and its structure was determined as follows.

The FAB-MS measurement of **1** gave a molecular ion $[M]^+$ at 551 *m/z* in good agreement with the mass calculated for $C_{24}H_{23}O_{15}$ (*m/z* 551.103) indicating the presence of each one molecule of 6-hydroxycyanidin, glucose, and malonic acid, respectively. The detailed structure of **1** was determined by the analysis of ^{1}H NMR, ^{1}H-^{1}H COSY and DIFNOE spectra of **1** (Table 1). The chemical shifts of five characteristic aromatic protons were assigned as shown in Table 1. However, the signal of H-6 disappeared in its ^{1}H NMR spectrum. The signal of an anomeric proton of glucose appeared at δ 5.43 (*d*, *J*=7.9 Hz). As all the assigned glucose protons have the vicinal coupling constants of *J*=7-12 Hz, indicating the glucose unit must be β-D-glucopyranose form. By irradiation at H-4 of 6-hydroxycyanidin, the observation of a negative NOE signal at H-1 of glucose indicated that glucose was bonded at OH-3 of 6-hydroxycyanidin. The protons of methylene in glucose moiety were shifted to a low magnetic field (δ 4.14 and 4.50), supporting that 6-OH of glucose is bonded with malonic acid. Therefore, the structure of **1** was determined to be 6-hydroxycyanidin 3-*O*-[6-*O*-(malonyl)-β-D-glucopyranoside] (Table 1, Figure 1), which is a new pigment.[9]

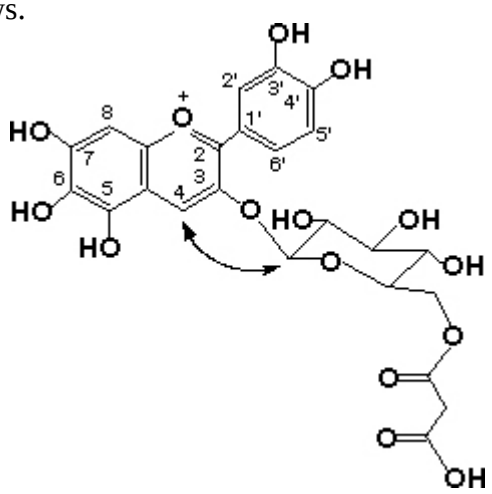

Figure 1. 6-hydroxycyanidin 3-malonyl-glucoside from *Alstroemeria* 'Tiara'. NOEs are indicated by arrows.

Table 1. ^{1}H NMR spectral data of 6-hydroxycyanidin 3-malonylglucoside(pigment 1) from the flowers of *Alstroemeria* `Tiara' (500 MHz, TFA-DMSO-*d*$_6$, 1:9 at 25°C, standard TMS)

6-hydroxycyanidin		glucose		malonic acid	
H-4	8.87 (1H, *s*)	H-1	5.43 (1H, *d*, *J*=7.9 Hz)	-CH$_2$-	3.20-3.50
H-8	7.10 (1H, *s*)	H-2	⎫		
H-2'	7.99 (1H, *d*, *J*=2.5 Hz)	H-3	⎬ 3.20-3.60		
H-5'	7.05 (1H, *d*, *J*=8.6 Hz)	H-4	⎭		
H-6'	8.21 (1H, *dd*, *J*=2.5, 8.6 Hz)	H-5	3.87 (1H, *m*)		
		H-6a	4.14 (1H, *dd*, *J*=8.0, 12.0 Hz)		
		H-6b	4.50 (1H, *brd*, *J*=12.0 Hz)		

Thus, we could determine the structure of a novel anthocyanin from the flowers of *Alstroemeria* 'Tiara'. Although the structure of a novel anthocyanin is relatively simple, the isolation of an acylated anthocyanin of 6-hydroxylated cyanidin would be of interest from the chemotaxonomical point of view.

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4. R. Nϕrbaek, L. P. Christensen, and K. Brandt, *Plant Breeding*, 1998, **117**, 63.
5. Pigment (**1**); UV λmax (0.1% HCl-MeOH) 513, 282 nm, E_{440}/Emax = 0.24; TLC Rf-values (x100) BAW (BuOH/AcOH/H_2O,4:1:2) 17, BuHCl (BuOH/2N-HCl, 1:1) 19, 1%HCl 7, AHW (AcOH/HCl/H_2O, 15:3:82) 19; FAB MS $[M]^+$ *m/z* 551; HPLC Rt (min) 20.61.
6. Pigment (**2**) (6-hydroxycyanidin 3-rutinoside); UV λmax (0.1% HCl-MeOH) 515, 284 nm, E_{440}/Emax = 0.23; TLC Rf-values (x100) BAW 14, BuHCl 16, 1%HCl 10, AHW 34; FAB MS $[M]^+$ *m/z* 611; HPLC Rt (min) 16.40. ^{1}H NMR (500 MHz, TFA/DMSO-d_6, 1:9): 6-hydroxycyanidin δ 8.81(1H, *s*, H-4), 7.06(1H, *s*, H-8), 7.94(1H, *d*, *J*=2.1 Hz, H-2'), 7.00(1H, *d*, *J*=8.9 Hz, H-5'), 8.16(1H, *dd*, *J*=2.1, 8.9 Hz, H-6'); Glucose, δ 5.34(1H, *d*, *J*=7.6 Hz, H-1), 3.49(1H, *t*, *J*=8.5 Hz, H-2), 3.37(1H, *m*, H-3), 3.20(1H, *t*, *J*=9.2 Hz, H-4), 3.66(1H, *m*, H-5), 3.42(1H, *m*, H-6a), 3.88(1H, *d*, *J*=10.1 Hz, H-6b); Rhamnose, δ 4.50(1H, *s*, H-1), 3.59(1H, *m*, H-2), 3.45(1H, *dd*, *J*=3.4, 9.5 Hz, H-3), 3.13(1H, *t*, *J*=9.5 Hz, H-4), 3.35(1H, *m*, H-5), 1.06(3H, *m*, -CH_3).
7. Pigment (**3**) (cyanidin 3-malonylglucoside); UV λmax (0.1% HCl-MeOH) 528, 280 nm, E_{440}/Emax = 0.25; TLC Rf-values (x100) BAW 29, BuHCl 56, 1%HCl 7, AHW 30; FAB MS $[M]^+$ *m/z* 535; HPLC Rt (min) 23.46.

8. Pigment (**4**) (cyanidin 3-rutinoside); UV λmax (0.1% HCl-MeOH) 529, 280 nm, E_{440}/E_{max} = 0.25; TLC Rf-values (x100) BAW 27, BuHCl 37, 1%HCl 17, AHW 47; FAB MS $[M]^+$ *m/z* 595; HPLC Rt (min) 19.16. ^{1}H NMR (500 MHz, TFA/DMSO-d_6, 1:9): Cyanidin δ 8.79(1H, *s*, H-4), 8.20(1H, *br d*, H-6'), 7.97(1H, *br s*, H-2'), 7.01(1H, *d*, *J*=8.5 Hz, H-5'), 6.88(1H, *br s*, H-8), 6.69(1H, *br s*, H-6); Glucose, δ 5.35(1H, *d*, *J*=7.7 Hz, H-1), 3.88(1H, *m*, H-6b), 3.63(1H, *m*, H-5); Rhamnose, δ 4.50(1H, *s*, H-1), 3.57(1H, *br s*, H-2), 3.37(1H, *m*, H-5), 1.03(3H, *m*, -CH_3).
9. J. B. Harborne, and H. Baxter, in *The Handbook of Natural Flavonoids, Vol. 2*, 1999, p. 1. Wiley, Chichester, New York, Weinheim, Brisbane, Singapore, Toronto.